Atty. Docket No.: **051058-030100** **PATENT**
(Formerly NUCL-002/01)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of:	Giordano, T et al.	Examiner:	LUNDGREN, J
Serial No.:	10/762,395		
Filed:	January 22, 2004	Group Art Unit:	1639
Entitled:	USE OF POST-TRANSCRIPTIONAL GENE SILENCING FOR IDENTIFYING NUCLEIC ACID SEQUENCES THAT MODULATE THE FUNCTION OF A CELL		
		Conf. No.:	4861

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Commissioner for Patents
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DECLARATION OF DR. CATHERINE PACHUK UNDER 37 C.F.R. 1.132

I declare:

1. I, Catherine Pachuk, hold the position of Senior Director at the Pfizer Research Technology Center of Pfizer Pharmaceuticals, Cambridge, MA.

2. I hold a Ph.D. Degree in Molecular Biology from The University of Pennsylvania. I am an author on more than 20 peer-reviewed literature publications. A copy of my Curriculum Vitae is attached. In particular, I have extensive post-filing experience in the area of eiRNA, or expressed inhibitory RNA, which takes advantage of the reduced stress response resulting from the expression of double-stranded inhibitory RNA in a target cell (see e.g., Romano et al., *Oncogene* 27:3857-3865 (2006); Snyder et al., *Antiviral Research* 80:36-44 (2008)). I have worked as a research immunologist for a number of years and have studied the cellular stress response in detail. In addition, I have published papers in peer-reviewed journals relating to immunology, such as the Journal of Immunology, Cellular Immunology, and the Journal of Infectious Diseases.

3. I am an inventor on the above-noted U.S. patent application.

4. I have read the Office Action issued February 3, 2010 in the above-noted patent application, and I understand that the Examiner has rejected claims 76, 79-82, 90, 91, 99, 100, 141, and 142 under 35 U.S.C. 112, first paragraph, as lacking enablement. I understand that the Examiner has concluded that the specification is only enabling for decreasing PSA expression in rhabdomyosarcoma cells with a 600nt expression cassette described in Example 10. The Examiner states:

> "it is clear that the stress response is unpredictable and that the expression vector used did not avoid the stress response, contrary to what Applicants appear to be suggesting based on Robbins."

5. I disagree with the Examiner's assertion that expressing a dsRNA molecule in the cell produces an unpredictable response, particularly regarding the induction of a cellular stress response. From my experience in the field of dsRNA mediated gene silencing, it is clear that when dsRNA molecules are administered intracellularly (e.g., by microinjection, electroporation, or expression from a vector) there is generally little to no stress response observed, while the same dsRNA molecules administered extracellularly (e.g., by liposome-mediated transfection) can induce a much stronger stress response. Intracellular administration or expression of dsRNA molecules can prevent a dsRNA molecule from interacting with cell surface Toll like receptors (TLRs) that activate a cellular immune response. In addition, extracellular administration of dsRNA molecules can result in the uptake of dsRNA molecules into endosomes. In the years since filing of the instant application it has become clear that dsRNAs which evade TLRs and uptake into endosomes provoke a reduced level of stress response compared to dsRNAs administered exogenously, which interact with one or both of these pathways. Thus, siRNA molecules that enter the cytoplasm directly (e.g., through electroporation or expression in the cell) avoid induction of inflammatory cytokines observed when the same siRNA molecule is administered through an endosome-mediated pathway that likely activates TLRs (e.g., by transfection of an siRNA). For example, the post-filing reference by Sioud et al. *J. Mol. Biol.* 348:1079-1090 (2005) (**Exhibit A**) demonstrates that electroporation of dsRNA largely avoids the stress response induced by transfection of the same dsRNA by liposomes – see, e.g., Figure 4(c) and (d), on page 1084. While the reference teaches that the degree of *stress response* is sequence dependent, it does not indicate that the *down-regulation of target gene expression* is

unpredictable, and it clearly shows that a method that avoids the TLRs and endosome uptake substantially avoids the stress response. Thus, the Sioud et al. reference confirms the teachings of the present specification and the predictability of the claimed invention.

6. I understand that Kenworthy et al. (2009, Nucleic Acids Research 37: 6587-6599) and Bauer et al. (2009, Gene Therapy 16: 142-147) have been cited to support the Examiner's argument that the intracellular expression of dsRNA molecules is unpredictable regarding the induction of a cellular stress response or lack thereof. However, the Kenworthy et al. reference states "The majority of the shRNAs that we use in the lab do not activate RIG-I expression and IFN signaling despite having essentially the same structure as sh-B971," (see e.g., page 6591, column 2, lines 1-3), "IFN activation depended on sequence," (see e.g., page 6588, column 1, lines 29-33, emphasis added), and "So far as we know, ours is the first report of IFN activation in the target cells by shRNAs delivered by lentiviral transduction" (see e.g., page 6597, column 1, lines 15-17). Kenworthy et al. clearly indicates that the sh-B971 shRNA does not behave in the same manner as the majority of shRNAs expressed intracellularly; that is, it induced a stress response when the majority of other shRNAs expressed intracellularly did not. The behavior of the sh-B971 shRNA is the exception and not the rule. One, or even several, exceptions do not render the technology unpredictable, especially where the scientist reporting the exception notes that it is unusual. Thus, Kenworthy et al. supports the conclusion that it is rare, at best, for a dsRNA effector, e.g., an shRNA, to elicit an appreciable interferon response when expressed in the cell.

7. In my opinion, there would be a reasonable expectation of success of using an intracellularly expressed shRNA to target a desired gene without appreciably inducing a stress response even in view of Kenworthy's teaching that the sh-B971 shRNA induced a stress response. My experience in the field of dsRNA mediated gene silencing supports the statements of Kenworthy et al. outlined above, in that only in rare cases does an intracellularly expressed dsRNA provoke a significant stress response. Expression of a dsRNA molecule in the cell very predictably reduces the level of stress response compared to that induced by administering the same dsRNA extracellularly. While some dsRNAs may indeed still cause a minor stress response when administered intracellularly, the level of the stress response is, in every instance of which I am aware, reduced compared to the stress response observed when that dsRNA is administered extracellularly. The discovery that one could reduce the level of an immune response by

expressing a dsRNA molecule inside the cell, rather than administering it to the outside of the cell, was an important discovery that helped to further the field of dsRNA- mediated gene silencing.

8. I understand that the Examiner has cited the following statements in the Bauer et al. reference in support of the conclusion of non-enablement:

> We show here that the induction of the interferon-response gene Oas1 by expression of first generation shRNA can be abolished by the introduction of the targeting sequence into a miR-30 backbone, whereby the modification of the passenger strand seems to be a crucial feature to avoid innate cellular immune response.

9. This statement is apparently being interpreted by the Examiner to teach that only expression in the miR-30 backbone would be able to avoid the stress response. However, this is a) incorrect on its face because intracellular expression has been demonstrated to avoid or reduce a stress response using a number of constructs that do not include a miR-30 backbone (see e.g., Sioud, M. et al. *J. Mol. Biol.* 348:1079-1090 (2005); **Exhibit A**), and b) is at odds with the Kenworthy et al. reference that states " So far as we know, ours is the first report of IFN activation in the target cells by shRNAs delivered by lentiviral transduction." That is, Kenworthy et al. indicates that induction of an interferon mediated stress response is rare when an shRNA is expressed in the cell. I note in particular that the Bauer et al. investigators did not measure the level of stress response when the shRNA is introduced from outside the cell, and consequently could not compare the level of stress response to that produced by the same shRNA expressed intracellularly. The results of Bauer et al. with the particular constructs they used cannot support a conclusion that the expression of their constructs does not result in reduced stress response relative to the stress response that would occur if the same shRNAs were introduced from outside the cell. While I acknowledge that it would require experimental data to absolutely confirm, based on my considerable experience in precisely this area, I would expect that the shRNA used in the Bauer et al. reference would induce a greater stress response when administered extracellularly than when expressed in the cell. While Bauer et al. may have found a method to further improve (i.e., decrease) the level of stress response induction when dsRNAs are expressed intracellularly, this result has no relevance to the invention as presently claimed.

10. In view of the above, it is my opinion that neither Bauer et al. nor Kenworthy et al. supports the conclusion regarding unpredictability drawn by the Examiner. In particular, even where there may remain some degree of stress response to dsRNAs expressed in the cell, in my opinion, based on considerable experience in this area, the level of that stress response will generally be considerably less than the response to the same dsRNA administered extracellularly.

11. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patents issued thereon.

Dec 1, 2010

Date

Catherine Pachuk

Catherine Pachuk, Ph.D.